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Detection of Hepatitis E Virus and Toll-Like Receptor 4 (rs4986790 and rs4986791) Genotypes Among A Sample of Hemodialysis Patients

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Abstract

Background Hepatitis E virus (HEV) is one of the prevalent nosocomial transmitted agents among patients on maintenance hemodialysis. Innate immunity's main actors, Toll-like receptors (TLRs), are able to identify the chemical patterns linked to pathogens.

Objective To investigate the seroprevalence of HEV in hemodialysis (HD) patients and study the TLR4 polymorphism (rs4986790 and rs4986791) in association with HEV in HD patients.

Methods One hundred and fifty patients on maintenance HD attending the HD centers of Al-Karama Hospital and Al-Yarmouk Teaching Hospital in the period from March to November 2021. Using enzyme-linked immunosorbent assay (ELISA) kit, serum samples were examined for the existence of anti-HEV (IgG and IgM) antibodies and conformation by using molecular technique quantitative reverse transcription polymerase chain reaction (qRT-PCR). The selected single nucleotide polymorphisms (SNPs) (rs4986790 and rs4986791) in TLR4 were amplified by using conventional PCR and then confirmed by sequencing their polymorphism.

Results Out of 150 hemodialysis patients, the seropositive result for HEV-IgG and IgM was 10 and 6, respectively. While 14 patients were positive by PCR. On the other hand, the result of IgM was negative for all control group. The analysis of TLR4 rs4986790 SNPs were 24 (80%) AA and 6 (20%) AG in patients while 6 (60%) AA and 4 (40%) AG in control group with insignificant difference. In addition, the TLR4 rs4986791 SNPs were 24 (80%) CC and 6 (20%) CT in patients while 6 (60%) CC and 4 (40%) CT in control group with insignificant difference.

Conclusion Patients undergoing HD are susceptible to HEV infection, the sero-prevalence of HEV in patients considered as risk factor. The genotypes of TLR4 SNPs (rs4986790 and rs4986791) have no significant association with HEV in HD patients.

Keywords HEV, seropositivity, hemodialysis, TLR4

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List of abbreviations: ELISA = Enzyme-linked immunosorbent assay, ERSD = End-stage renal disease, HD = Hemodialysis, HEV = Hepatitis E virus, HLA = Human leukocyte antigen, PCR = Polymerase chain reaction, qRT-PCR = Quantitative reverse transcription polymerase chain reaction, SNPs = Single nucleotide polymorphisms, TLRs = Toll-like receptors

Introduction

The single-stranded RNA virus known as hepatitis E virus (HEV), which causes hepatitis E, belongs to the genus Orthohepevirus in the family Hepeviridae [(1)]. Although the fecal- oral route is the predominant method of HEV transmission, alternative methods include hemodialysis (HD)

is a part of blood transfusions, and organ donation are some other possible routes of HEV transmission [2]. Patients receiving maintenance HD frequently contract HEV, one of the common nosocomial transmitted diseases. The parenteral transmission of HEV and immunocompromised state of continuous HD patients are the causes of this elevated exposure risk. Therefore, HEV infection is a risk for HD patients [3].

This susceptibility is confirmed by the high prevalence of HEV infection among HD patients around the world [4]. However, HEV prevalence among HD patients in various regions can also be influenced by the levels of safety precautions in HD centers and the frequency of HEV in the population [5].

Despite the fact that HEV infection is typically moderate and self-limiting in its clinical manifestation, people with chronic kidney disease, particularly those receiving HD, often have severe bouts of the infection [6]. However, its significance, HD patients are not frequently checked for HEV in HD centers, particularly in endemic countries [3].

Toll-like receptors 4 (TLR4) is involved in the pathogenesis of several viral diseases, including hepatitis B, C, and E [7]. The single nucleotide polymorphisms (SNPs) rs4986790 (A>G, Asp299Gly) and/or rs4986791 (C>T, Thr399Ile) in TLR4 are well-known. Receptor hypo-responsiveness, not receptor expression, is correlated with increased TLR 4 expression (at protein and gene level) and reduced cytokine response upon stimulation of peripheral blood mononuclear cells with lipopolysaccharides in HEV infected patients. These alter the molecule's extracellular domain that found in the fourth exon [8].

This study focused on identifying of HEV, and estimate the TLR4 polymorphism as useful in revealing of disease progression and treating of HD patients.

Methods

One hundred and fifty patients on regular HD (79 males and 71 females) from two dialysis centers in Al-Karama Hospital and Al-Yarmouk Teaching Hospital were enrolled in this study. The control group consisted of 150 apparently healthy individuals from the donor and blood transfusion center and volunteers. The samples were collected from March to November 2021.

Hepatitis E virus-IgG and IgM antibodies were detected by using HEV IgG, IgM enzyme-linked immunosorbent assay (ELISA) kit (Acon, USA). The whole blood was further tested for detection of HEV RNA using quantitative reverse transcription polymerase chain reaction (qRT-PCR) (SacaceBiotechnologies, Italy). Hepatitis E virus RNA was extracted from the samples using the guanidiniumisothiocyanate (GIT) method and a modified proteinase K (PK) method (BioinGentech, Italy).

To synthesize single-stranded cDNA from total RNA using the cDNA Reverse Transcription kit and the primers that used (R 5'-CCCTTRTCYTGCTGMGCATTCTC-3'), F(5'-ATTATGCYCAGTAYCGRGTTG -3') [9].

Polymorphisms of TLR4 (rs4986790 and (rs4986791) was performed by PCR (F:5'-TCTGGCTGGTTTAGAAGTCCA-3', R:(5'-ATTGCCAGCCATTTTCAAG-3') [10] and Sanger sequencing method, by Macrogen Corporation of Korea's ABI3730XL automated DNA sequencer.

After receiving the results through email, knowledgeable software was used in data analysis. All patients and the control group have given their consent. The Institutional Review Board (IRB), College of Medicine at Al-Nahrain University gave approval to this study.

Statistical analysis

Statistical package for the social sciences (SPSS) Software v19.0 was used to examine the data. The bivariate analysis was carried out using the chi-square test in order to identify the risk factors associated with the seropositivity of microorganisms. P-values lower than 0.05 were regarded as statistically significant.

Results

Out of 150 HD patients, 79 (52.7%) were males and 71 (47.3%) were females, while in control

group there were 80 (53.3%) males and 70 (46.7%) were females. The median age of patients and controls were 41.35 and 42.50 years, respectively with no significant difference (Table 1).

Table 1. Age and sex of patients and control groups

Parameter		Patients	Controls	P value
Age (yr)	Median (5-95 percentile)	41.35 (21-63)	42.50 (20-65)	>0.05
Sex	Female No. (%)	71 (47.3%)	70 (46.7%)	>0.05
	Male No. (%)	79 (52.7%)	80 (53.3%)	

Regarding the result of RT-PCR, there were 14 (9.3%) of patients positive for HEV, while all the control group were negative, with highly significant difference (Table 2).

Table 2. Detection of hepatitis E virus by quantitative reverse transcription polymerase chain reaction

HEV PCR		Patients	Control
Positive	No. %	14 9.3%	0 0.0%
Negative	No. %	136 90.7%	150 100%
Total	No. %	150 100.0%	150 100%
P value		≤0.001**	
Odd ratio (95%CI)		2.103	1.86-2.38

** Highly significant

Of the 150 HD patients 10 (6.7%) were seropositive for anti- HEV IgG antibody and 6 (4.0%) had anti-HEV IgM antibody, while among control group all samples were negative for anti-HEV IgG and IgM antibodies (Table 3 and 4).

Table 3. Anti-HEV IgG seropositivity rates in study groups

Anti-HEV IgG		Patients	Control
Positive	No. %	10 6.7%	0 0.0%
Negative	No. %	140 93.3%	150 100%
Total	No. %	150 100%	150 100%
P value		≤0.001**	

** Highly significant

Table 4. Anti-HEV IgM seropositivity rates in study groups

Anti-HEV IgM		Patients	Control
Positive	No.	6	0
	%	4.0%	0.0%
Negative	No.	144	150
	%	96.0%	100%
Total	No.	150	150
	%	100%	100%
P value		0.015*	

* Significant

The majority of HD patients seropositivity of HEV were in the age groups 51-60 years with insignificant difference (Table 5).

Table 5. Association of HEV seropositivity and quantitative reverse transcription polymerase chain reaction results with age groups

Age groups		HEV PCR		Anti-HEV IgG		Anti-HEV IgM	
		Positive	Negative	Positive	Negative	Positive	Negative
≤ 30 years	No.	4	30	3	31	1	33
	%	11.80%	88.20%	8.80%	91.20%	2.90%	97.10%
31-40 years	No.	2	28	3	29	1	31
	%	6.70%	84.80%	10.10%	87.90%	0.00%	93.90%
41-50 years	No.	1	33	0	34	0	33
	%	2.90%	97.10%	0.00%	100%	2.90%	97.10%
51-60 years	No.	5	28	4	27	2	30
	%	15.20%	93.30%	12.00%	90.00%	2.90%	100.00%
61-70 years	No.	2	17	0	19	2	17
	%	10.50%	89.50%	0.00%	100%	10.50%	89.50%
Total	No.	14	136	10	140	6	144
	%	9.30%	90.70%	6.70%	93.30%	4.00%	96.00%
P value		0.48		0.188		0.417	

Concerning the sex, there were 8 (10.1%) females and 6 (8.5%) males positive for HEV by PCR, while 6 (8.5%) females and 4 (5.1%) males were positive anti-HEV IgG, and 3 (4.2%) female and 3 (3.8%) males were positive for anti-HEV IgM by ELISA with no significant difference (Table 6).

According to the analysis of TLR4-rs4986790 SNPs using Sanger sequencing this polymorphism appeared in only two genotypes in both patients and control group, these were AA and AG the frequency of the heterozygous genotype (AG) was equal in patients and control group (50% in each them) (P=0.393).

At allelic level, the frequency of mutant allele (allele G) also was equal in patients and control group (50% in each them) (Table 7).

TLR4 rs4986791 similar to TLR4 rs4986790, this polymorphism also had only two genotypes CC and CT (Table 8).

Table 6. Association of HEV seropositivity with sex

Sex		HEV PCR		Anti-HEV IgG		Anti-HEV IgM	
		Positive	Negative	Positive	Negative	Positive	Negative
Female	No.	8	65	6	65	3	68
	%	10.10%	91.50%	8.50%	91.50%	4.20%	95.80%
Male	No.	6	71	4	75	3	76
	%	8.50%	89.90%	5.10%	94.90%	3.80%	96.20%
Total	No.	14	136	10	140	6	144
	%	9.30%	90.70%	6.70%	93.30%	4.00%	96.00%
P value		0.725		0.307		0.607	

Table 7. Frequency of genotypes and alleles of TLR-4 rs4986790 in study groups

TLR4rs4986790			Study groups		P value
			Patients	Controls	
Genotype	AA	No.	24	6	0.232^{NS}
		%	80.00%	60.00%	
	AG	No.	6	4	
		%	20.00%	40.00%	
Allele	A allele	No.	54	16	0.257^{NS}
		%	90.00%	80.00%	
	G allele	No.	6	4	
		%	10.00%	20.00%	

NS: Non-significant

Table 8. Frequency of genotypes and alleles of TLR-4 rs4986791 in study groups

TLR4 rs4986791			Study groups		P value
			Patients	Controls	
Genotype	AA	No.	24	6	0.232^{NS}
		%	80.00%	60.00%	
	AG	No.	6	4	
		%	20.00%	40.00%	
Allele	A allele	No.	54	16	0.257^{NS}
		%	90.00%	80.00%	
	G allele	No.	6	4	
		%	10.00%	20.00%	

NS: Non-significant

As well as there was no significant association between HEV results by ELISA and PCR with TLR4 SNPs (table 9 and 10).

Table 9. Association of genotypes and alleles of TLR4 rs4986790 with HEV in patients' group

TLR4 rs4986790		HEV PCR Positive	HEV PCR Negative	Anti-HEV IgG Positive	Anti-HEV IgG Negative	Anti-HEV IgM Positive	Anti-HEV IgM Negative	Total
AA	No.	7	17	4	20	2	22	24
	%	29.17%	70.83%	16.67%	83.33%	8.33%	91.67%	100%
AG	No.	3	3	2	4	1	5	6
	%	50.00%	50.00%	33.33%	66.67%	16.67%	83.33%	100%
A	No.	17	37	10	44	5	49	54
	%	31.48%	68.52%	18.52%	81.48%	9.26%	90.74%	100%
G	No.	3	3	2	4	1	5	6
	%	50.00%	50.00%	33.33%	66.67%	16.67%	83.33%	100%
P value		0.393		0.586		0.484		

Table 10. Association of genotypes and alleles of TLR4 rs4986791 with HEV in patients' group

TLR4 rs4986791		HEV PCR Positive	HEV PCR Negative	Anti-HEV IgG Positive	Anti-HEV IgG Negative	Anti-HEV IgM Positive	Anti-HEV IgM Negative	Total
CC	No.	7	17	4	20	2	22	24
	%	29.17%	70.83%	16.67%	83.33%	8.33%	91.67%	100%
CT	No.	3	3	2	4	1	5	6
	%	50.00%	50.00%	33.33%	66.67%	16.67%	83.33%	100%
C	No.	17	37	10	44	5	49	54
	%	31.48%	68.52%	18.52%	81.48%	9.26%	90.74%	100%
T	No.	3	3	2	4	1	5	6
	%	50.00%	50.00%	33.33%	66.67%	16.67%	83.33%	100%
P value		0.393		0.586		0.484		

Discussion

This case control study revealed insignificant difference in median age among patients and control group, because the ages of control group were selected according to patients' group. Most studies worldwide showed that older ages patients (≥50 years) were more likely to have infections by microorganisms. However, some studies did not find such association [11].

The current study revealed that 6.7% of patients were seropositive of anti-HEV IgG antibodies while among control group, all samples were negative with significant difference.

On the other hand, the IgM anti-HEV seropositive rates in hemodialysis patients were 4%, while all samples of the control group were negative with no significant difference.

The HEV seroprevalence reported among HD patients were (39.6%) in Egypt [12]. Alavian et al. [13] found anti-HEV IgG antibodies in 28.3% of HD patients in Isfahan, Iran, as opposed to 9.9% in their control group. Similar to this, Argentinian dialysis patients had considerably

greater seroprevalence than control group (10.2% and 4.3%, respectively) [14].

Other study from England showed that HD patients (36.8%) have a considerably greater seroprevalence of anti-HEV IgG than control group (18.8%) [15]. However, other studies revealed that HD patients' HEV prevalence is not appreciably higher than that of healthy people. The same HEV isolates were discovered in the patient's serum and the transfused viremic blood in a study by Mitsui et al. [16] that demonstrated how an HD patient became positive for HEV RNA by transfusion of HEV-viremic blood one month after HD onset.

This enormous regional variation in the prevalence of HEV seroprevalence may be brought on by variations in the severity of safety precautions and preventive measures implemented in HD facilities, as well as variations in the burden of HEV infection in the general population, risk factors, routes of HEV transmission, and the state of public health and hygiene in various regions. But some of this heterogeneity might be attributable to variations in the specificity and sensitivity of ELISA kits, research period, sample size, timing of sampling, length of disease, and socio-demographic features of the study population in other studies [17].

Other risk factor is the potential for HEV transmission in hemodialysis patients through tainted blood transfusions and heparin. The older individuals have frequent exposure to the outside environment, including contaminated food and water, and this frequent exposure increases the chances of contracting the virus. The virus dose may not be sufficient to cause infection, but it can induce the immune system to produce IgG antibodies against HEV. Anti-HEV IgG seropositive of hemodialysis patients in the current study was higher with age range 51-60 yrs. Numerous studies have found a relationship between older age and increased HEV seropositivity [18].

It is possible that more parenteral exposures or cumulative exposure over time are to blame for this rising incidence with age [19].

Infections with HEV frequently progress asymptomatically or without typical symptoms, resulting in a seroconversion of IgG antibodies that is unremarkable clinically. Consuming raw or undercooked meat, drinking tap water, and receiving blood products other than red blood cells in transfusions have all been mentioned as contributing factors to HEV infection [20].

The nutritional state is another element (micronutrient deficiencies). A high burden of viral diseases and micronutrient deficiencies have been found to contribute to immunologic compromise, including decreased mucosal immunity and dysregulated cytokine production. The immune system may become compromised as a result of this imbalance, raising the risk of HEV infection [21].

It has been documented that infectious HEV has been identified in a variety of sources, including animal feces, sewage water, inadequately-treated water, contaminated shellfish, and animal meat. In-house breeding of domesticated animals or close proximity to human houses are a few factors that may contribute to increased rates of HEV infection among women [22].

Numerous studies have searched for genetic factors associated with HD risk, the role of human leukocyte antigen (HLA)-A2 in Iraqi Arab patients and HLA-B35 in Iraqi Kurdish patients could be considered as highly significant risk factors for end-stage renal disease (ESRD) [23].

This study showed no association of TLR4 rs4986790 and rs4986791 polymorphism with HEV infection. An earlier study found that the TLR4 polymorphism was linked to high viral loads, delayed antiviral therapy, and patients with HCV-induced hepatocellular cancer [24]. Additionally, a different study found a strong correlation between the TLR4 polymorphism and the development of HEV illness [25].

In conclusion: Patients undergoing hemodialysis are susceptible to HEV infection, the seroprevalence of HEV in patients considered as risk factor. The genotypes of TLR4 SNPs (rs4986790 and rs4986791) have no significant association with HEV in HD patients.

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Author contribution
Dr. Salih: Contributed to the design and implementation of the research also wrote the manuscript. Dr. Abbas: Conceived of the presented idea and supervised the project.

Conflict of interest
The authors declare there is no conflict of interest.

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